

**Supplement S 5. Identification of the influence of system-specific parameters on the frequency response analysis results.**

Calculation of initial state values for the differential equations by approximating the steady state values. Firstly, six auxiliary variables were calculated, in which [antagonist] and [dopamine] refer to the applied concentration of antagonist and dopamine in the simulated experiment, of which only the latest phase is simulated.

Auxiliary variables:

$$RLi = \frac{R_{tot} * [antagonist]}{K_D antagonist * \left( 1 + \frac{[dopamine]}{\frac{k_{off} dopamine + RR}{k_{on} dopamine}} + \frac{[antagonist]}{K_D antagonist} \right)}$$

$$RD = \frac{R_{tot} * [dopamine]}{\frac{k_{off} dopamine + RR}{k_{on} dopamine} * \left( 1 + \frac{[dopamine]}{\frac{k_{off} dopamine + RR}{k_{on} dopamine}} + \frac{[antagonist]}{K_D antagonist} \right)}$$

$$a = \frac{k_3 * k_4}{k_5}$$

$$b = k_2$$

$$c = -k_{0max} * \frac{RLi^h}{RLi^h + LFR_{50} * R_{tot}} - k_1 * \left( 1 - \frac{RD^h}{RD^h + DAFR_{50} * R_{tot}} \right)$$

$$d = \frac{k_4}{k_5}$$

Initial states of the differential equations:

Antagonist concentration: [antagonist] –  $RLi$

Dopamine concentration: [dopamine] –  $RD$

Receptor-antagonist complex:  $RLi$

Receptor-dopamine complex:  $RD$

cAMP concentration:  $\frac{-b + \sqrt{b^2 - 4ac}}{2a}$

PDE concentration:  $d * \frac{-b + \sqrt{b^2 - 4ac}}{2a}$

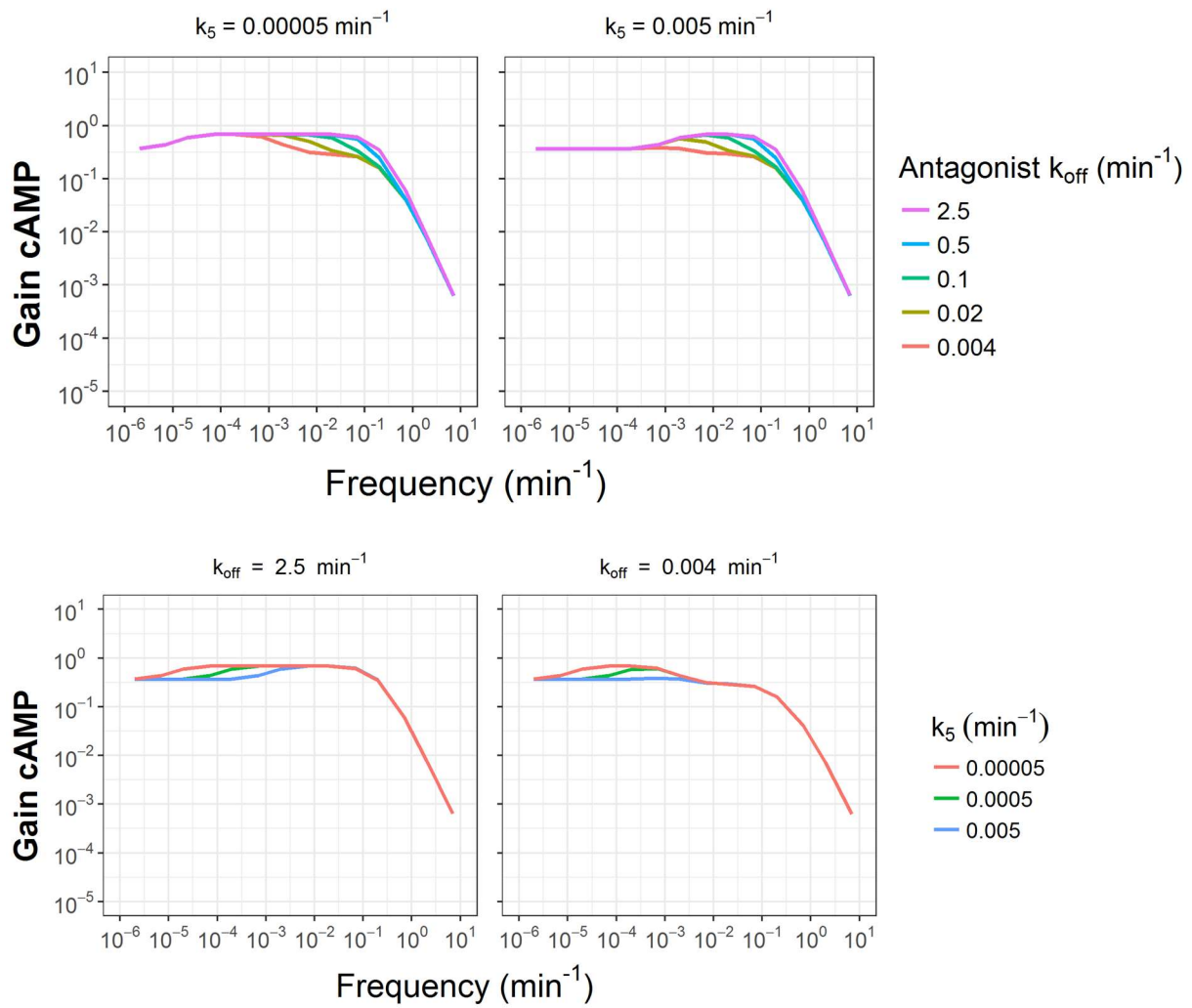


Figure S 5. Frequency response analysis for 3 different active PDE turnover rate constants and 5 different antagonist  $k_{\text{off}}$  values. The upper plots show the influence of the antagonist  $k_{\text{off}}$  for two different active PDE turnover rate constants, and the lower plots show the influence of the active PDE turnover rate constant for two different  $k_{\text{off}}$  values. The input signal was a sine wave of free dopamine with an amplitude of 10nM and baseline of 20 nM, at the frequencies indicated on the x-axis. At each active PDE turnover rate, 5 different antagonist  $k_{\text{off}}$  values were simulated, which are represented by the different line colors. The  $k_{\text{on}}$  values were changed simultaneously with  $k_{\text{off}}$ , which means that the  $K_D$  was constant at 6.93 nM. The antagonist concentration was 14 nM, the  $\text{LFR}_{50}$  was 1.03 and all system-specific parameters were identical to Table 3.

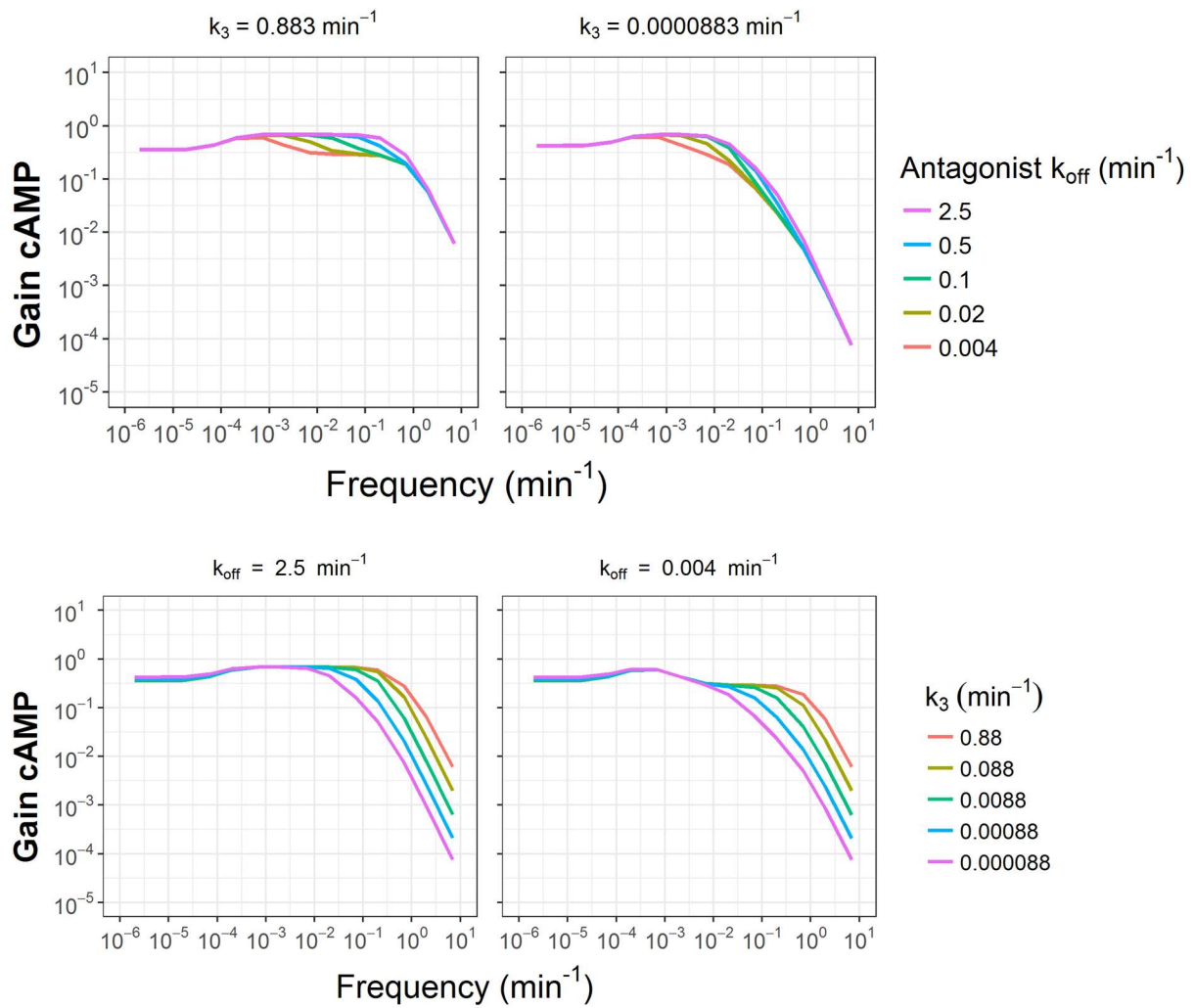


Figure S 6. Frequency response analysis for 5 active PDE-dependent cAMP turnover rate constant ( $k_3$ ) values and 5 antagonist  $k_{\text{off}}$  values. The upper plots show the influence of the antagonist  $k_{\text{off}}$  for two different active PDE turnover rate constants, and the lower plots show the influence of the active PDE-dependent cAMP turnover rate constant for two different  $k_{\text{off}}$  values. The input signal was a sine wave of free dopamine with an amplitude of 10nM and baseline of 20 nM, at the frequencies indicated on the x-axis. At each cAMP turnover rate, 5 different antagonist  $k_{\text{off}}$  values were simulated, which are represented by the different line colors. The  $k_{\text{on}}$  values were changed simultaneously with  $k_{\text{off}}$ , which means that the  $K_D$  was constant at 6.93 nM. The antagonist concentration was 14 nM, the  $\text{LFR}_{50}$  was 1.03 and all system-specific parameters were identical to Table 3.

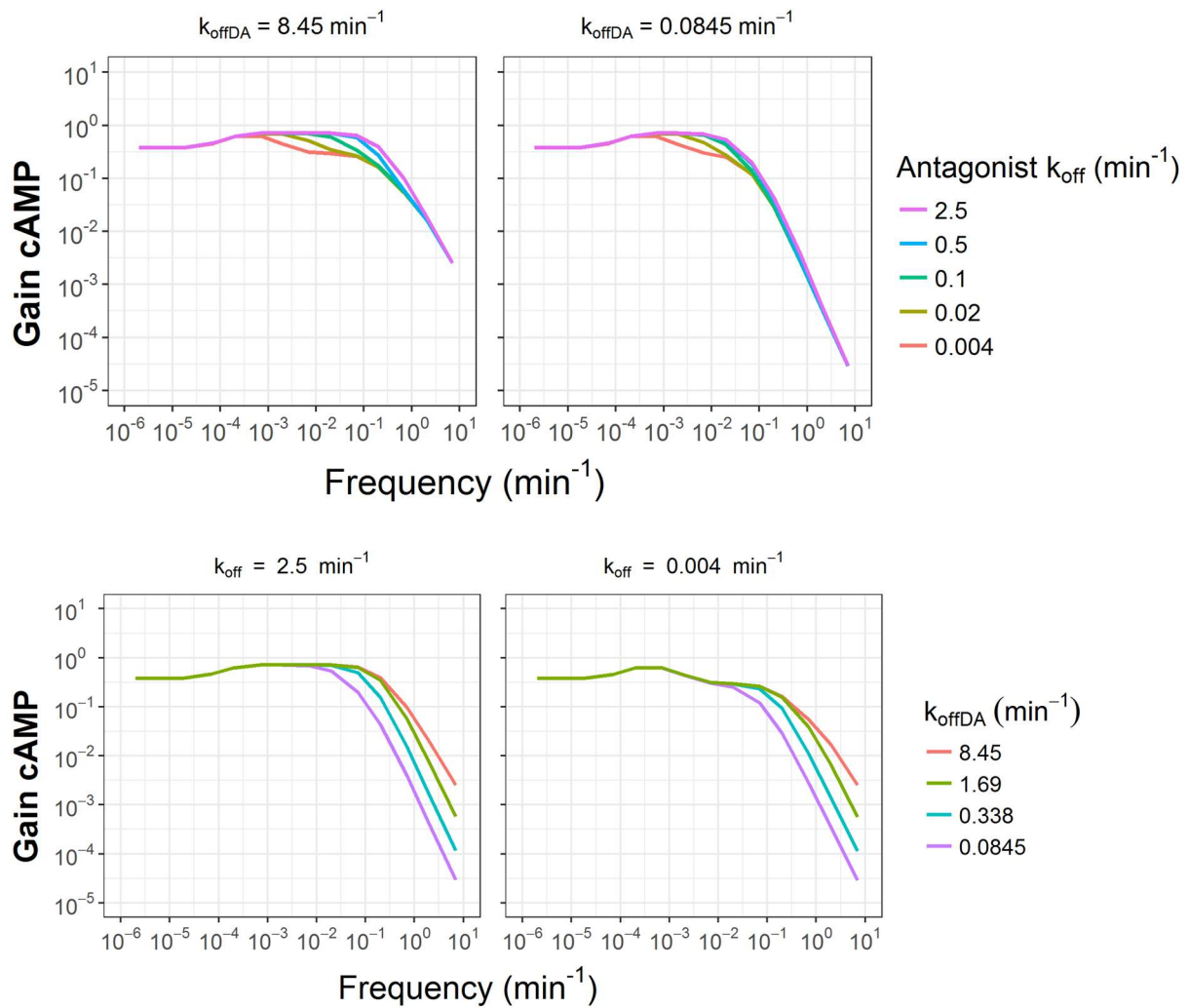


Figure S 7. Frequency response analysis for 4 different dopamine-receptor  $k_{\text{off}}$  values and 5 different antagonist  $k_{\text{off}}$  values. The upper plots show the influence of the antagonist  $k_{\text{off}}$  for two different active PDE turnover rate constants, and the lower plots show the influence of the dopamine  $k_{\text{off}}$  for two different antagonist  $k_{\text{off}}$  values. The input signal was a sine wave of free dopamine with an amplitude of 10nM and baseline of 20 nM, at the frequencies indicated on the x-axis. At each dopamine dissociation rate constant, 5 different antagonist  $k_{\text{off}}$  values were simulated, which are represented by the different line colors. The  $k_{\text{on}}$  values were changed simultaneously with  $k_{\text{off}}$ , which means that the  $K_D$  was constant at 6.93 nM. The antagonist concentration was 14 nM, the  $\text{LFR}_{50}$  was 1.03 the receptor recycling rate constant was switched to 0 and all other system-specific parameters were identical to Table 3.

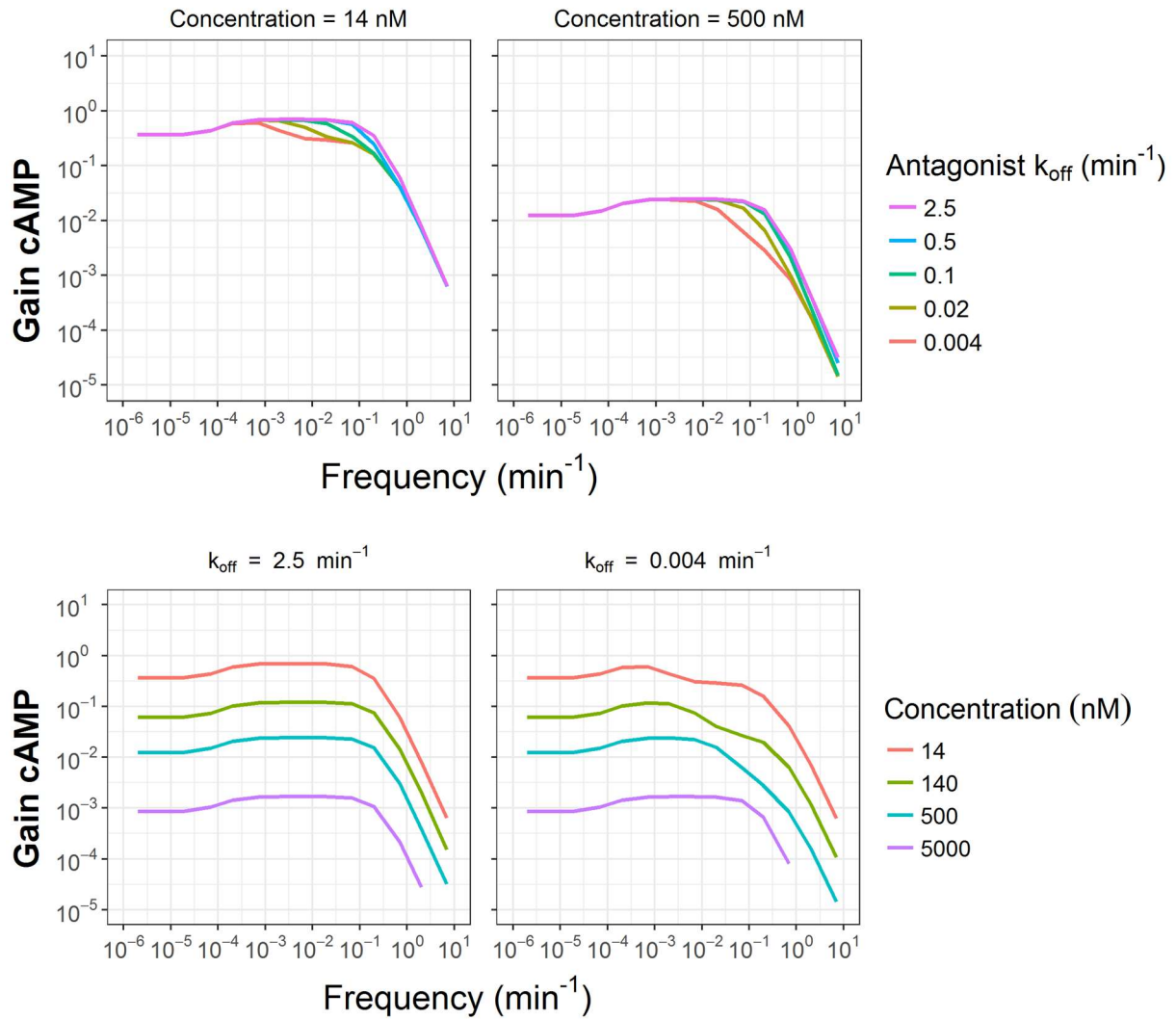
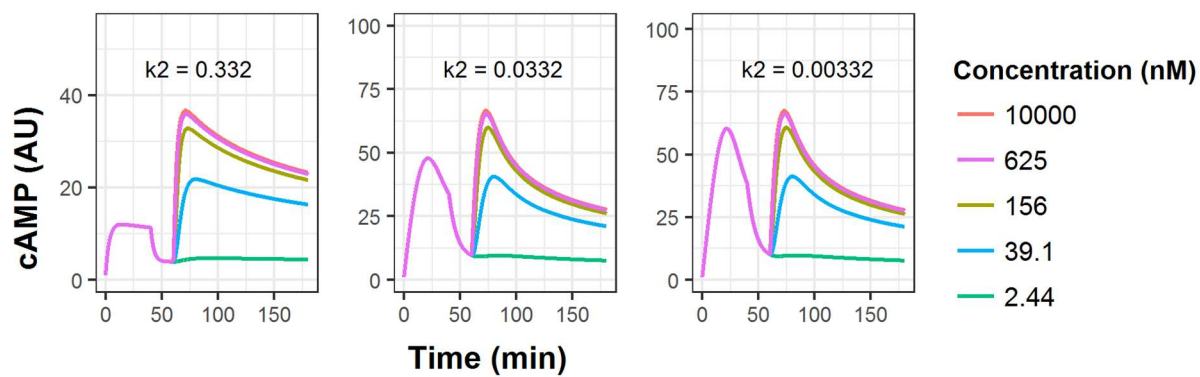
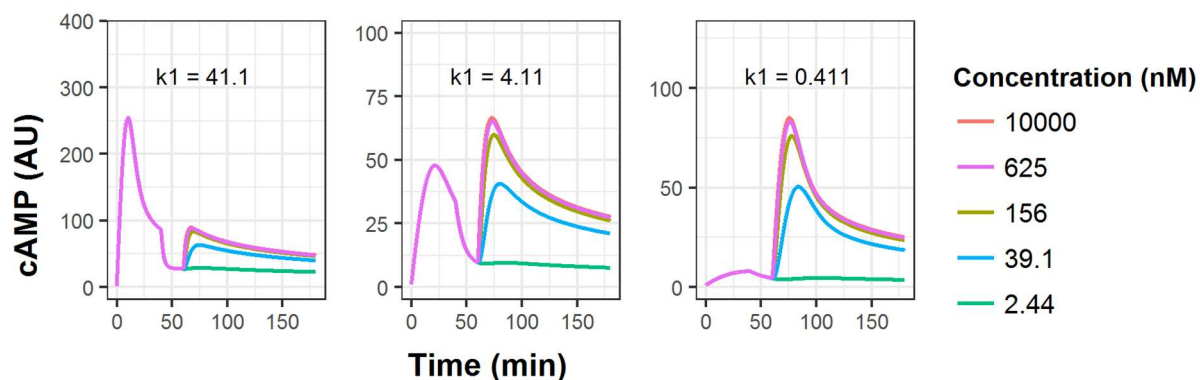
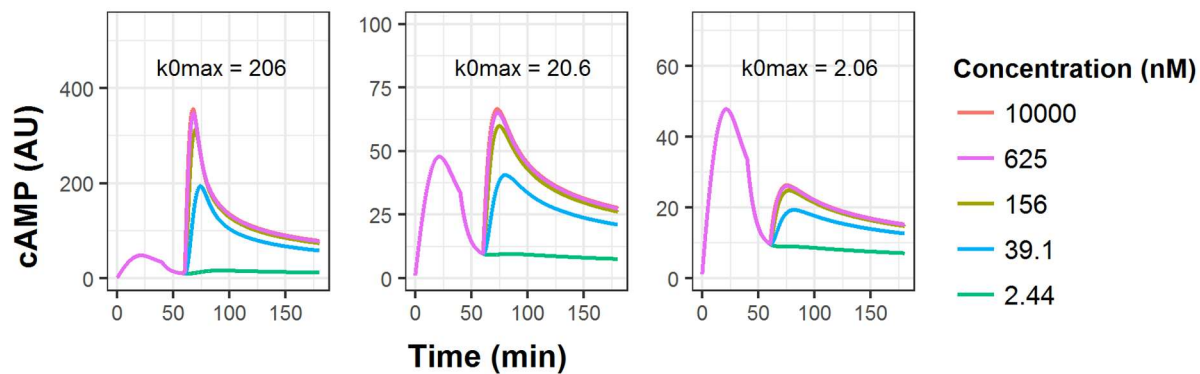
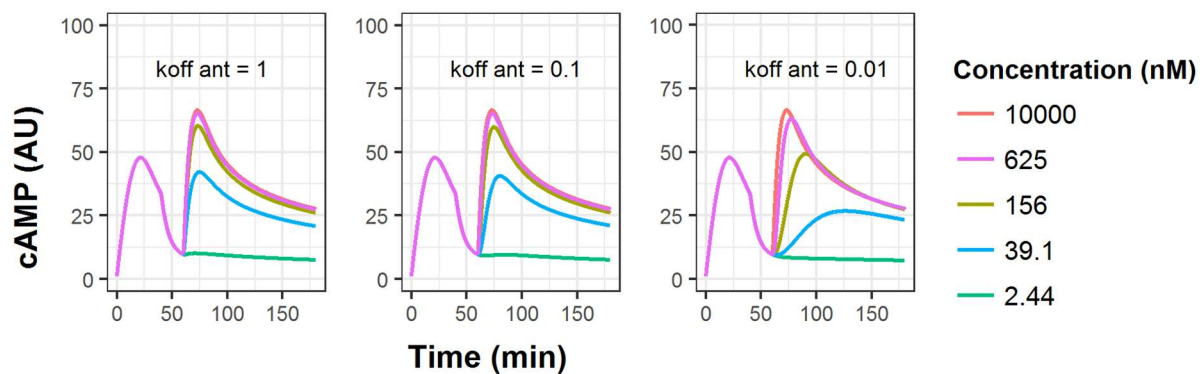
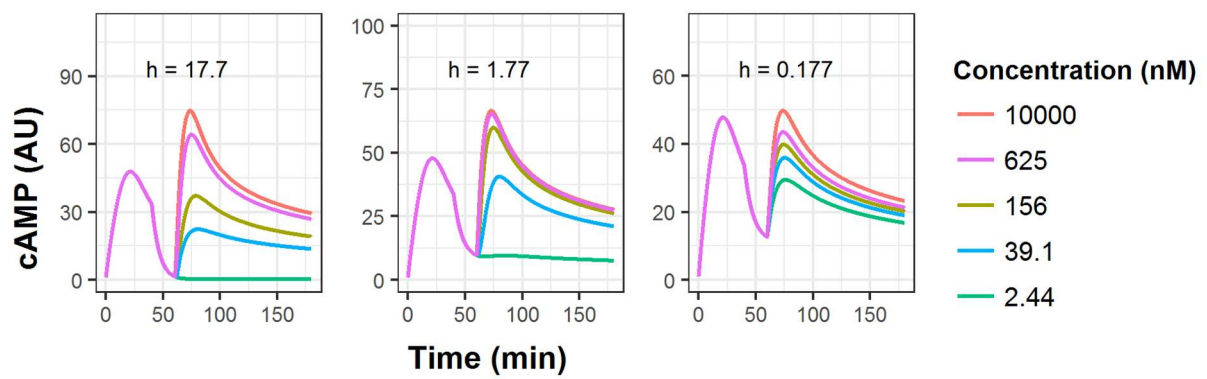
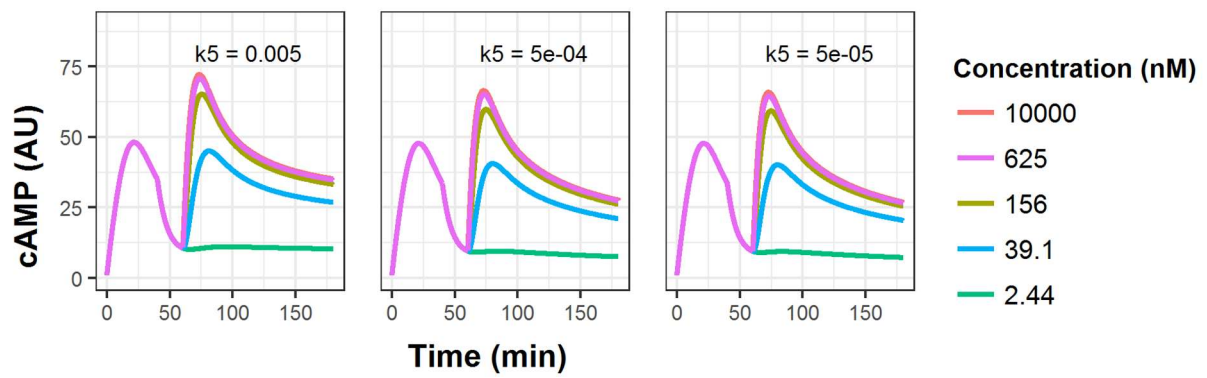
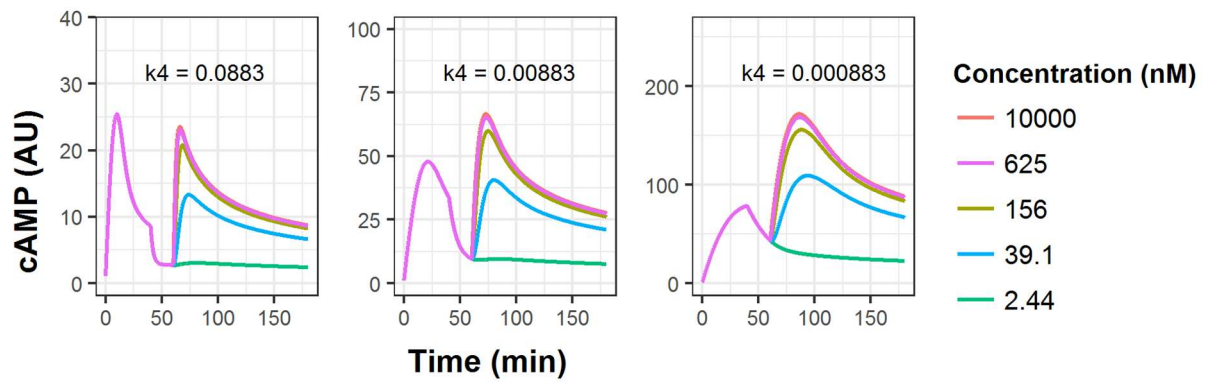
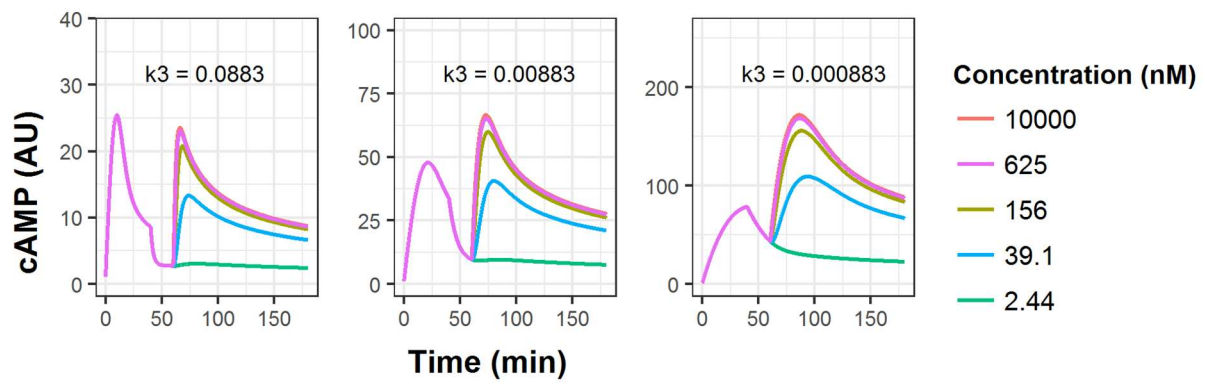
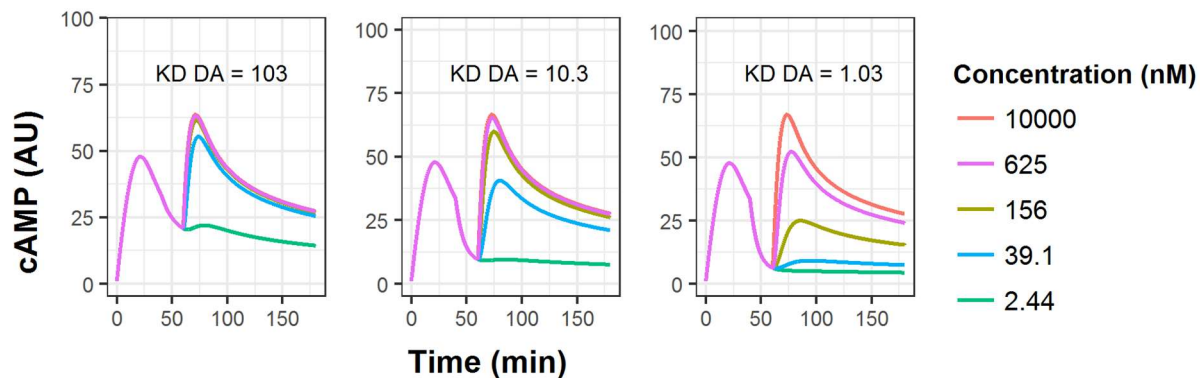
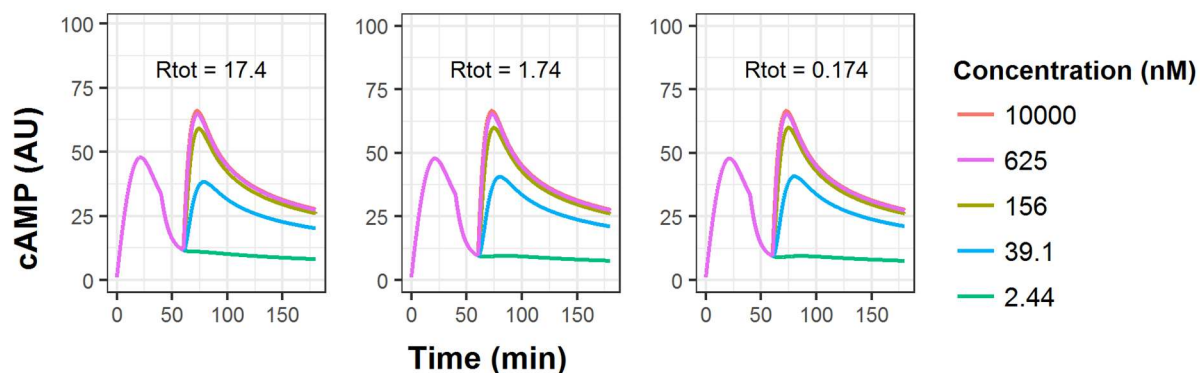
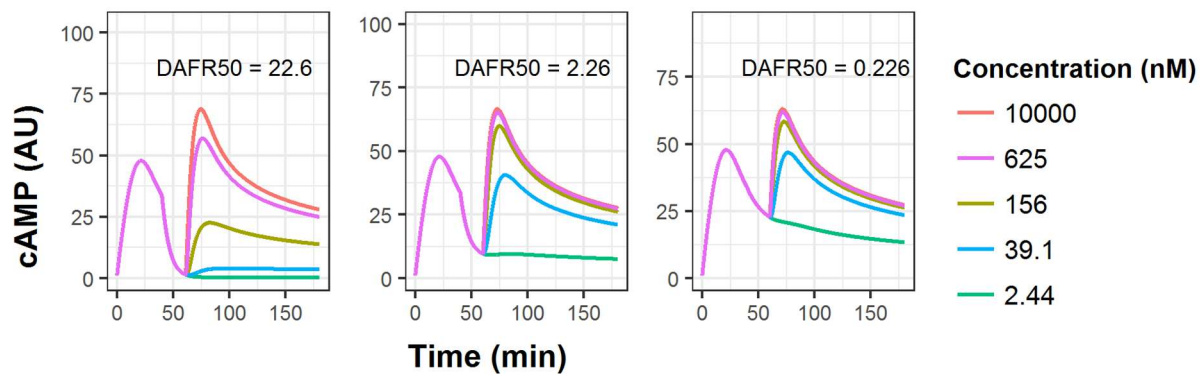
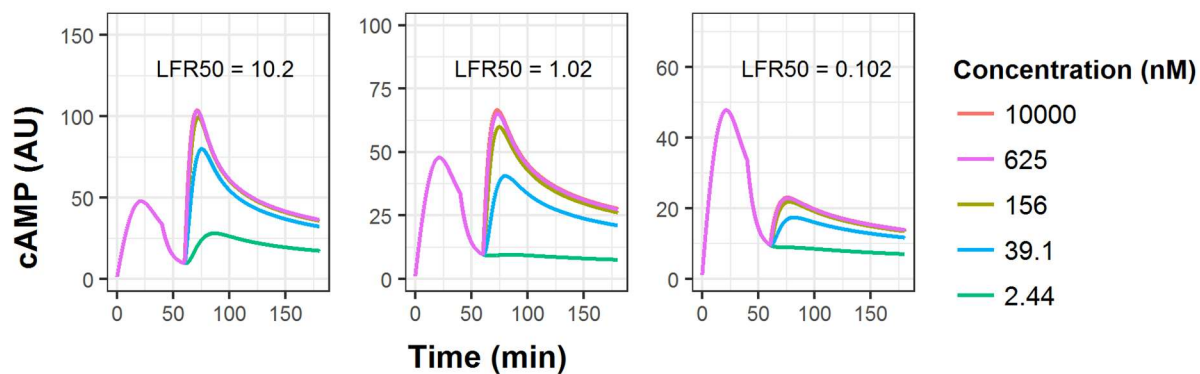


Figure S 8. Frequency response analysis for 4 different antagonist concentrations and 5 different antagonist  $k_{\text{off}}$  values. The upper plots show the influence of the antagonist  $k_{\text{off}}$  for two different active PDE turnover rate constants, and the lower plots show the influence of the antagonist concentration for two different antagonist  $k_{\text{off}}$  values. The input signal was a sine wave of free dopamine with an amplitude of 10 nM and baseline of 20 nM, at the frequencies indicated on the x-axis. At each antagonist concentration, 5 different antagonist  $k_{\text{off}}$  values were simulated, which are represented by the different line colors. The  $k_{\text{on}}$  values were changed simultaneously with  $k_{\text{off}}$ , which means that the  $K_D$  was constant at 6.93 nM. The antagonist concentration was 14 nM, the  $\text{LFR}_{50}$  was 1.03 and all system-specific parameters were identical to Table 3.







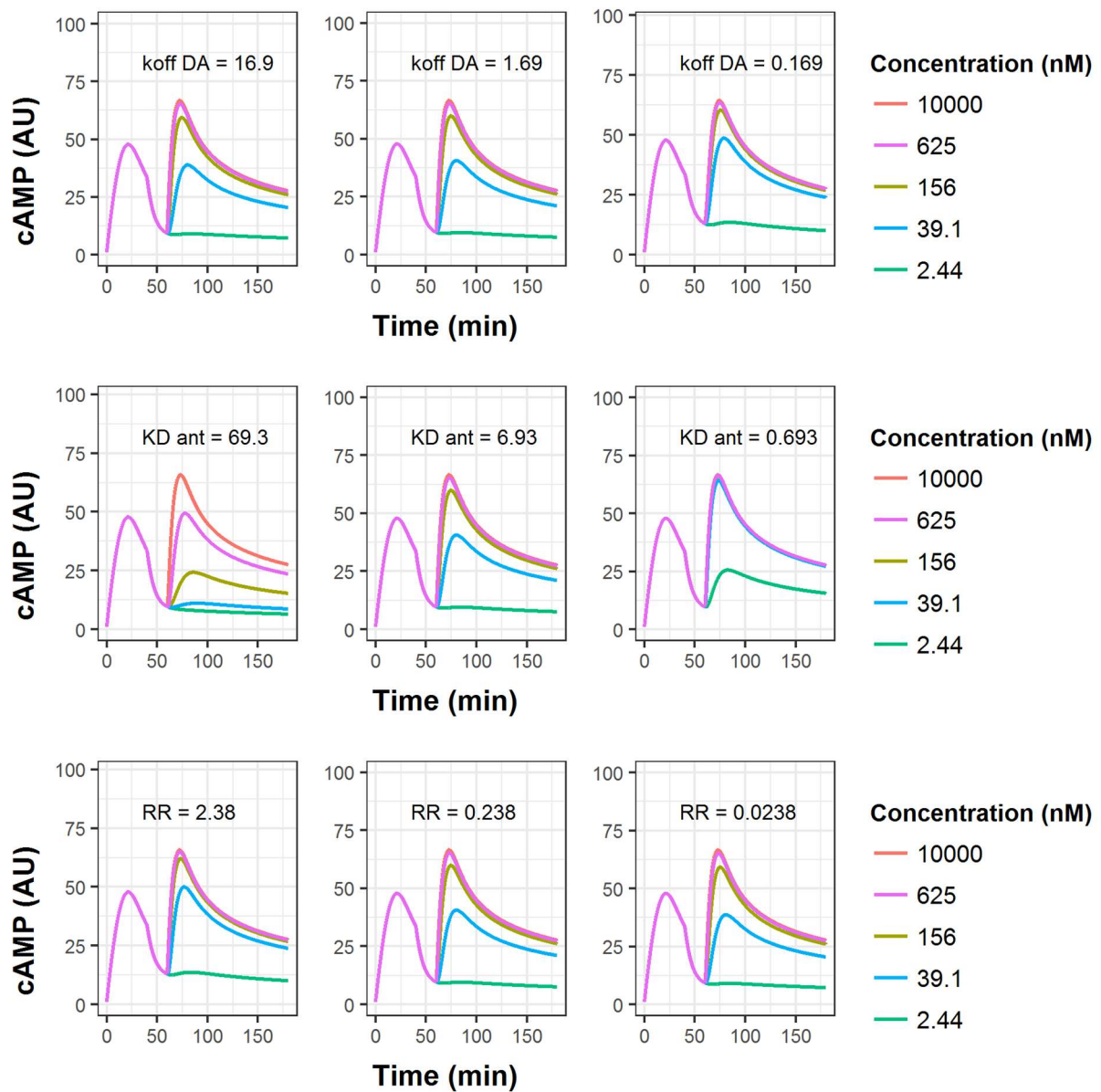


Figure S 9. Sensitivity analysis for 5 different antagonist concentrations (line colours) and for a 10-fold increase and decrease of each parameter from Table 3, the antagonist  $K_D$ ,  $k_{off}$  and the  $LFR_{50}$  (panels). The middle panels are the same in the whole figure, representing the parameters in Table 3, an antagonist  $K_D$  value of 6.93 nM, an antagonist  $k_{off}$  value of 0.1  $\text{min}^{-1}$  and an  $LFR_{50}$  value of 1.02. The y-axis can change between the different panels.